1. BACKGROUND AND RATIONALE

An estimated one million Americans are infected with the human immunodeficiency virus type 1 (HIV-l), a human retrovirus and causative agent of the acquired immunodeficiency syndrome (AIDS) (l-4). Current estimates of numbers of infected individuals, projections of numbers of people who will become newly infected over the next several years, and the enormous amount of resources often required in the care and management of people with HIV infection, imply that the epidemic will make even greater demands on already-limited services.

Therapeutic strategies for intervening in HIV disease include antiretroviral therapy, treatment and prophylaxis of opportunistic infections, anti-tumor therapy, immunomodulator therapy, and immunologic restoration. Zidovudine (AZT), an inhibitor of reverse transcriptase, was the first antiretroviral drug to be approved by the Food and Drug Administration (FDA) for AIDS. It has been shown in a randomized, placebo-controlled trial to prolong the lifespan of HIV-infected patients. The effect is limited, however, by the development of drug resistance in most patients (5). The second generation reverse transcriptase inhibitors, Didanosine (ddI), zalcitabine (ddC), and stavudine (d4T), have been approved for use alone or in combination with zidovudine (6). More recently the nucleoside analogs, lamivudine (3TC) and stavudine (d4T), have also been approved for treatment of HIV infection (7). A new class of anti-viral drugs, HIV protease inhibitors, has recently entered clinical trial in HIV infected subjects. Results show encouraging anti-viral activity as measured by dramatic suppression of HIV plasma RNA and prolongation of life, especially when used in combination with reverse transcriptase inhibitors (8-12). On this basis, three protease inhibitors (saquinavir, ritonavir, and indinavir) have now been approved for use in the United States.

Although the number of FDA-approved antiretroviral agents is growing, there are numerous practical limitations to their use. Toxic side effects prevent some patients from taking these drugs in combination for extended periods. Moreover, the available evidence suggests that continuous combination drug therapy is necessary to maintain viral suppression. Drug resistant strains of virus are likely to evolve under the selection pressure of antiretroviral agents if suppression of viral replication is not complete. Despite the major advances in treating HIV disease that have occurred in the past five years, efficacious, less toxic therapies with novel mechanisms of action are still greatly needed.

Much experimental data have suggested that the CD8+ T cell response may represent the major and earliest immune response to HIV infection. While a high frequency of HIV antigen-specific CD8+ T cells are found in the peripheral blood of asymptomatic HIV seropositive patients, a decline in HIV-specific CTLs is observed in patients as they progress to later stages of the disease (13,

14). This suggests that a breakdown of the host cell mediated immune response is associated with progression to AIDS. *In vitro* studies have shown that HIV-specific CD8+ T cells exhibit cytolytic activity toward HIV-infected targets and inhibit HIV replication in lymphocyte cultures (15). These results suggest that adoptive transfer of HIV-specific CTLs may have potential as immunotherapy for HJV-infected individuals.

Adoptive immunotherapy of viral infection using antigen-specific T cells has been studied by several investigators. Riddell *et al.* have explored this strategy using an isolated T cell clone with HLA-restricted antigenic specificity for CMV (16, 17). CMV-specific CD8+ T cells isolated from MHC-identical bone marrow donors were expanded *ex vivo* and administered to 14 allogeneic bone marrow transplant recipients. Recovery of CMV-specific CTL activity was seen in each case and adoptively transferred CTL persisted *in vivo* for up to 12 weeks. No patient developed CMV disease. However, cellular immunity to CMV declined in patients deficient in CMV-specific CD4+ T cells, suggesting that CD4+ helper T cell function was necessary for the persistence of the transferred CD8+ CTL. In a similar study, Brenner *et al.* administered donor-derived EBV-specific CD8+ and CD4+ T cells, genetically marked with the *neo*^{r} gene, to 6 recipients of T cell depleted allogeneic bone marrow transplants (18, 19). These patients are at high risk for developing EBV-associated lymphoproliferative disorders post-transplant due to uncontrolled EBV-driven B cell proliferation and transformation. EBV-specific cellular immune responses were restored in all 6 patients following adoptive T cell transfer, and EBV-related post-transplant lymphoproliferative disorder was eradicated in one patient. Gene-marked CD4+ and CD8+ T cells responsive to *in vivo* or *ex vivo* challenge with EBV persisted *in vivo* for as long as 18 months post infusion. These data provide further evidence that antigen-specific CD4+ cells may provide growth factors that facilitate the persistence and expansion of both CD4+ and CD8+ cells *in vivo.* These encouraging studies validate the strategy of adoptive transfer of antigen-specific T cells for reconstitution of cellular immunity to CMV and EBV. Adoptive immunotherapy of HIV infection has been attempted by Riddell *et al.* using autologous HIV *gag*-specific CTL clones retrovirally transduced with a chimeric suicide gene construct (HyTK; hygromycin phosphotransferase-thymidme kinase fusion gene) (20). Five of 6 patients developed CD8+ class I MHC-restricted CTL responses specific for epitopes derived from the novel HyTK protein and eliminated the transduced cells upon repeat challenge. No efficacy data have been reported to date. The encouraging results with adoptive T cell therapy of EBV and CMV infection hold promise for the efficacy of this approach in the setting of HIV infection.

Rather than isolating and expanding rare T cell clones with MHC-restricted antigenic specificity, a method has been developed to rapidly generate large numbers of HIV-specific, MHC-unrestricted T cells using retroviral-mediated gene transfer to insert an HIV-targeting gene (CD4-zeta) into primary CD8+ and CD4+ T cells (21). These genetically marked cells can be expanded *ex vivo,*

resulting in the rapid generation of large numbers of HIV-specific T cells. CD4-zeta is a genetically engineered, MHC-unrestricted, chimeric receptor composed of the zeta subunit of the T cell receptor (the cytoplasmic domain involved in signal transduction) fused to the transmembrane and extracellular domains of human CD4 (which targets the gp120 component of HIV expressed on the surface of HIV-infected cells).

Preclinical studies have shown that CD4-zeta gene-modified T cells proliferate and secrete cytokines in response to target cells transfected with HIV-*env*. CD4-zeta bearing T cells also mediate the *in vitro* killing of *env*-expressing tumor targets as well as T cells infected with primary and laboratory isolates of HIV-l. This cytolysis is specific, as no killing of "innocent bystander" cells or MHC class II-expressing cells has been seen. Furthermore, cytolysis is not inhibited by soluble anti-gpl20 antibodies or free HIV antigen (21). In follow up studies, CD4-zeta gene-modified CD8+ T cells were shown to inhibit viral replication in HIV-infected T cell cultures in vitro. Despite bearing a novel receptor with the CD4 extracellular domain, gene-modified CD8+ T cells were incapable of supporting productive infection following exposure to 10 primary clinical isolates of HIV-l including a one low-passage, AZT-resistant, syncytia-inducing strain (unpublished data).

A Phase I/II trial of adoptive immunotherapy with syngeneic CD4-zeta gene-modified CD8+ T cells in HIV-infected identical twins is currently underway. In this study, CD8+ lymphocytes are isolated from the HIV seronegative twin, the CD4-zeta gene is introduced, and gene-modified CD8+ cells are then purified and expanded *in vitro* in the presence of a high concentration of IL-2 (700 IU/mL). The length of time in culture has ranged from 50-107 days. No significant toxicity was noted during the initial phase I dose escalation period with infusion of up to 10^{10} CD8+ gene-modified T cells. As of December 30, 1996, 20 patients have received multiple infusions of 10^{10} gene-modified cells. Cell infusions have been well tolerated and no serious toxicity due to cell therapy has been noted. Persistence of CD4-zeta gene-modified cells in the circulation has been detected as long as 36 weeks post-infusion and gene-modified cells have been detected in lymphoid tissue in 3 of 4 patients.
Preliminary studies demonstrate persistent expression of CD4-zeta RNA in 2 of 2 patients by RT-PCR analysis for 8 and 18 weeks post-infusion, respectively. Preliminary efficacy data, however, do not indicate any consistent changes in CD4+ T cell count or plasma viral load related to gene-modified CD8+ T cell infusions.

A phase II study of autologous CD4-zeta gene-modified CD8+ T cells in HIV-infected subjects has been initiated. In the pilot phase of this study, HIV infected patients with CD4+ counts of 50-500 and plasma viral loads of 1,000-10,000 copies/ml will receive 3 infusions of 2-3 x 10^9 gene-modified CD8+ T cells. Two patients have been infused as of December 30, 1996; no results are yet available.

While these studies have been underway, research efforts at Cell Genesys, Inc. have focused on cell culture and processing improvements designed to deliver a T cell product with optimal *in vivo* activity. Due to a possible deficiency of HIV-specific CD4 "helper" function in HIV infected subjects receiving gene-modified CD8+ T cells alone, preclinical studies of CD4-zeta gene-modified CD4+ and CD8+ cells have been performed. CD4-zeta transduced CD4+ T cells were shown to proliferate and secrete IL-2 in response to stimulation through the CD4-zeta receptor using immobilized anti-CD4 antibodies in conjunction with CD28 costimulation. CD4-zeta gene-modified CD4+ T cells also demonstrated specific cytolytic activity against *env*-expressing tumor targets with cytolysis equivalent to that seen with bulk gene-modified CD4+ and CD8+ peripheral blood lymphocytes (unpublished data).

In addition to co-transduction of CD4+ and CD8+ T cells, cell culture improvements include T cell stimulation with antibodies to CD3 and CD28 co-immobilized on immunomagnetic beads, a lower dose of IL-2 in the cell culture medium (50-200 IU/mL), and a shorter length of time in culture. Stimulation of T cells with immobilized antibodies to CD3 and CD28 has been shown to promote the *in vitro* survival and proliferation of CD4+ T cells as well as to inhibit HIV infection of these cells (22). Analysis of CD4-zeta gene-modified T cells cultured using CD28 co-stimulation has verified the CD4 proliferative and HIV inhibitory effects of this cell culture method (unpublished data).

In addition to manipulation of the transduced T cell population and cell culture conditions, co-administration of exogenous IL-2 in conjunction with gene-modified T cells may enhance the survival and anti-viral activity of adoptively transferred T cells. A recent study by Kovacs *et. al.* showed that continuous intravenous infusion of IL-2 at doses ranging from 6-12 million IU/day for 5 consecutive days every 8 weeks in HIV infected individuals with CD4+ counts > 200/mm^3 resulted in an approximately two-fold increase in peripheral CD4+ T cell counts (23). In the presence of effective anti-retroviral therapy, no significant change in HIV plasma viremia was noted. IL-2 dosed in this range was reasonably well tolerated with expected side effects of fatigue, myalgias, fever, headache, diarrhea, hyperbilirubinemia, and vascular leak. With the exception of fatigue, the other side effects were noted in < 25% of patients. At a dose of 9 million IU/day, approximately 25% of patients required dose reduction secondary to toxicity following successive cycles of IL-2, whereas at a dose of 6 million IU/day < 10% of patients required further dose reduction. The increase in CD4+ T cell counts was similar at doses ranging from 6-12 million IU/day given for 5 consecutive days. In patients with CD4+ T cell counts of 50-200/mm^3 (excluded from this trial), IL-2 given at these doses is similarly well tolerated as long as viral replication is effectively suppressed with combination anti-retroviral therapy (H.C. Lane, personal communication). These data demonstrate the safety and efficacy of IL-2

administration in HIV-infected individuals and provide the rationale for studying the co-administration of IL-2 with adoptively transferred gene-modified T cells.

The purpose of this current pilot study is to evaluate the safety and anti-viral activity of autologous CD4-zeta gene-modified CD4+ and CD8+ T cells, produced using the more efficient and physiologic cell culture conditions described above, in HIV-infected subjects with CD4+ T cell counts above $50/mm^3$. In addition, subjects will be randomized to receive gene-modified cells infused with or without the addition of exogenous IL-2 to determine whether co-administration of IL-2 enhances the survival or anti-viral activity of adoptively transferred T cells.